Phase behavior of C18 monoglyceride in hydrophobic solutions

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We apply a set of different techniques to analyze the physical properties and phase transitions of monoglycerides (MG) in oil. In contrast to many studies of MG in water or aqueous systems, we find a significant difference in the phase structure at different concentrations and temperatures. By adding small quantities of water to our base MG/oil systems we test the effect of hydration of surfactant head-groups, and its effect on the phase behavior. The phase diagrams are determined by calorimetry and their universal features are recorded under different conditions. Two ordered phases are reported: the inverse lamellar gel phase and the sub-alpha crystalline gel phase. This sequence is very different from the structures in MG/water; its most striking feature is the establishing of a 2D densely packed hexagonal order of glycerol heads in the middle of inverse lamellar bilayers. Rheology was examined through temperature scans to demonstrate the gelation phenomenon, which starts from the onset of the lamellar phase during the cooling/ordering process.

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1 Introduction

Monoglyceride (MG) is a lipid molecule consisting of a single fatty acid chain linked to a glycerol head.[1,2] MG variants are distinguished by the length of carbon chain and here we focus on a particularly common surfactant labeled C18. Because of the strong emulsifying property, MG is widely used in personal care products, cosmetics and in food industry to tailor product properties in a specifically manner.[3-7] A lot of manufactured oil-based food products such as chocolate, cakes and creams contain fat (triglyceride) as a structuring material, which raises obesity issues. In order to reduce the fat in the oil-based products, one of the strategies is to achieve the structuring of oil without fat, by exploiting the liquid crystalline phase of long-chain saturated MG in hydrophobic solvents.[8] A particular feature is the use of MG/oil mixtures as healthy substitute for butter.[9,10] Apart from specific industrial applications, MG/oil systems are a part of generic materials, which form a percolating network of structured aggregates to rend the solvents inside.[11] For these reasons the detailed knowledge of phase behavior and micro-structures of MG/oil systems are important in terms of both scientific reasons and the technological implementations.

MG aqueous systems have been studied systematically for many years. The liquid-crystal and fully crystalline structures of pure MG was firstly described by Larsson in 1966[12] and was later reviewed by Small (1986),[13] Larsson (1994),[14] and Krog (2001).[15] Its polymorphic behavior is well known, with the following phase sequence on cooling: isotropic fluid, lamellar and alpha-crystalline phases.[11,16-18] When polar lipids are mixed with water, between the gelation temperature and the Krafft temperature the water has a strong affinity to the polar glycerol groups and will penetrate the polar sheets, forming a lamellar liquid crystalline phase which contains the lamellar ordering with disordered carbon chains.[16] Below the Krafft temperature T_K the alkyl chains are partially frozen and a hydrated mesomorphic phase, named the alpha-crystalline gel (L_β), is formed.[19] This phase is characterized by a single X-ray reflection in wide angles, corresponding to a short spacing of 4.18Å, which shows hexagonal packing.[8,13,15]

The alpha-crystalline phase is metastable and eventually ages into the anhydrous MG crystal, named the beta-crystalline state (often referred to as the "coagel"), which has a higher melting point and is characterized by a number of distinct wide-angle X-ray reflections, with the strongest line corresponding to the spacing 4.5-4.6Å.[13,15] A coagel state of pure MG, and equivalently in below the Krafft demixing temperature is due to hydrogen bonds establishing within head groups in bilayers, which in turn lead to a further crystallization of aliphatic tails.[20] On a long time-scale of aging, the D- and L- isomers of chiral MG gradually separate within crystalline bilayers, leading to more dense packing and full expulsion of water. Sedimentation of solid in this phase then takes places.[20,21]

Comparing with aqueous MG systems, the phase behaviors of MG in a fully hydrophobic solvent is much less studied. In this case some rheological and storage properties, and the network features have been reported by Shimoni et al,[9,10,22] but due to the absence of the phase diagram and confident structure description the connection between the molecular arrangement and macroscopic observations is weak and need systematic analysis. In fact, many authors in this field continue to assume that the phase diagram of MG in oil is the same, or similar, as in water, whereas it is clear that hydrogen-bonding patterns of aggregated glycerol groups would

be very different.[9,10] There are many examples of the use of ternary MG/water/oil systems; however, the presence of water will dominate the phase behavior of aggregating MG.[8,23,24] Some of our X-ray results reported here are similar to those of Marangoni et al,[8] but once again our present work is different since we deliberately conducted studies with no water present.

In the case of MG/water, the molecules pack in usual hydrophilic lamellae, with alkyl chains packing inside the bilayer, which in turn are surrounded by water, binding to the glycerol heads by hydrogen bonds. In the lamellar phase the head groups are in thermal motion, and the aliphatic tails inside the bilayer are also molten. It is a well-studied liquid-crystalline system. In particular, considering the hydrophobic chains of each monolayers as an extended polymer brush, it is clear that normal pressure on the bilayer would not change the structure of chains in such a brush.[25] In contrast, in the hydrophobic environment, the inverse lamellar bilayer structure will form, with aliphatic tails on the outside the bilayer, also in the molten extended-brush conformation. However, the hydrophobic head groups are now compressed in the middle of the bilayer by the effective pressure. The high entropy of the lamellar phase is taken up by the random alkyl chains, while the compressed glycerol heads inside adopt a two-dimensional close-packed conformation (2D hexagonal lattice). Therefore a single wide-angle reflection at 4.17Åcorresponding to the closest distance of approach of glycerol heads in a plane is observed.[15] The second, nearby peak at 4.11Åis the other characteristic distance in this bilayer of closely packed glycerol heads, i.e. the distance between the neighboring heads in different layers. The phase transition from the isotropic to this inverse-lamellar phase would still be a reversible first order phase transition.

The most dramatic difference of MG solutions in oil (in contrast to MG/water, or most other surfactants in oil) is that the inverse lamellar phase macroscopically behaves as an elastic gel, unlike the complex-fluid rheological behavior of ordinary flexible lamellae. In most other surfactant solutions, and certainly in aqueous MG systems, the gelation only occurs at lower temperatures due to crystallization of phases. We assert that the origin of this inverse-lamellar gelation is in the 2D hexagonal ordering of glycerol heads inside the bilayer that sets in from the moment the bilayer is formed. In hydrated bilayer lamellae this is not possible, and most other surfactants that form inverse bilayers in oil have different size ratio between the heads and the tails, and also do not achieve this 2D crystallization on dense-packing.

The next, lower-temperature phase transition occurs on cooling the inverse lamellar phase below its crystallization point. The hexagonal lateral packing of extended molten chains in the dense brush transforms into a sub-alpha crystal form, which has orthorhombic chain packing, characterized by strong X-ray short spacing at 4.17Åand several spacings from 4.06 to 3.6Å.[15] These dimensions are represented in the Xray scattering pattern we obtain below T_K, in the phase that we continue to call "sub-alpha crystal" to preserve the analogy with the well-studied phase of MG in water.[8,11,16–18,23,24] In aqueous MG systems this phase is also found, but much lower in the phase sequence, essentially when the bilayer lamellae with crystallized tails parallel-pack so close together that the glycerol heads on their are able to establish a 2D lattice in the contacting planes. In our water-free system, the 2D hexagonal order sets in before the crystallization of aliphatic tails, and so sub-alpha phase is adjacent to the inverse lamellar phase.

In this paper different techniques were applied to provide a comprehensive set of measure-

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ments for understanding MG/oil systems. Firstly, in order to distinguish the MG/oil systems from water-containing systems, we demonstrate that the presence of water, even in small quantity (0.5% w/w), gives a significant change of phase behavior. We examine MG/oil systems over the whole range of surfactant concentrations, but specifically focus on the range 0 to 10%, because this is the most important for region for applications. The phase diagram was obtained by calorimetry with high resolution. Two universal transitions, gelation and crystallization, were indicated in different conditions (different types of oil, varying cooling/heating rate). The crystallographic structure in different phases was determined by high-resolution X-ray diffraction. We find two unique ordered states: the inverse lamellar phase (with hexagonal head packing) and "sub-alpha" crystalline state. The rheological properties in different phases and across phase transition boundaries have been studied to give a phenomenological description of mechanical properties. A particular feature, again in stark contrast with water-MG solutions is that the inverse lamellar phase rheologically behaves as a stiff gel, due to its 2D lattice of closely packed heads inside each bilayer.

2   Experimental details

Distilled saturated MG were purchased from Palsgaard A/S (Denmark). The sample contained 92% monoglyceride C18 and 5% C16. The remaining 3% consisted mainly of diglycerides and small amounts of triglycerides. Pure MG C16 (99+% pure) had been prepared by chemical synthesis in our laboratory[26] to compare with the commercial materials (which are always impure to some degree). Two hydrophobic solvents were used in the study: natural hazelnut oil, and n-tetradecane as a pure model oil. The hazelnut oil was obtained from Provence (France) where this variety contains approximately 80% oleic and 20% linoleic acids with low quantities of MG.[27] This oil crystallizes at a temperature below $-23°$C. Before testing the hazelnut oil was heated to $120°$ for several hours to keep its drying condition. n-tetradecane was purchased from BDH chemicals Ltd (Poole, UK) with a quoted purity of 99%; the freezing point of this model hydrophobic solvent is $5.9°$C. The mixtures of MG and oil were stored on a heating plate at a constant temperature of $100°$C, in a dessicator with a magnetic stirrer.

Later on, in the discussion of our results, one may question how important was the small impurity of the MG in the sequence of phase transformations. We are clear that this is not important at all. To test this point we have separately investigated the phase transitions of pure MG C16 in n-tetradecane. There are small quantitative changes (reported and discussed later in the text), but the generic feature of two consecutive phase transitions, and the structures of ordered phases, remains universal.

Heat exchange involved in a phase transition yields exothermic or endothermic peaks that were recorded in a differential scanning calorimeter (DSC) experiment. From these measurements the transition temperatures can be estimated with good resolution. A Perkin-Elmer power-compensated Pyris 1 DSC equipped with an Intracooler 2P was used. To focus on the interested phase transitions, samples were heated to $100°$C, held for 1 min, cooled to $0°$C at a specified rate, and then reheated to $100°$C at the same rate. The experiment was then repeated at a different heating/cooling rate with the same sample.

The X-ray scattering patterns were recorded at different temperatures for 10% (w/w) mono-glyceride/oil sample. Small-angle X-ray diffraction (SAXS) was performed using a copper rotating anode generator (Rigaku-MSC Ltd) equipped with X-ray optics by Osmic Ltd. Before recording the X-ray diffraction, the samples were heated to 70°C for 5 min to erase the structure memory and then cooled down to a given temperature. The distance between the small angle detector to the sample was set to 300mm, giving the maximum resolution of 3.36Åat the edge of the diffraction pattern. Samples, of thickness 1mm, were held between mica sheets of 0.1mm thick (supplied by Goodfellow, Cambridge, UK) and an aluminium plate. A metal substrate plate was used to ensure accurate heat transfer to the sample. The temperature was controlled by a home-made chamber and verified by a thermocouple. The bilayer lamellar spacing and crystallographic lengths of crystalline phases were calculated from the diffraction patterns using Bragg scattering analysis.[28]

Rheological measurements were staged on a strain-controlled rheometer (Rheometrics PHYS-ICA MCR501, Anton German) connected to a water-bath temperature control, an acceptable source since our working range was between 70°C to 20°C. A plane-plane sensor design of annular gap 0.4mm was utilized to ensure a constant shear rate in the total volume of the liquid. The sample used in the rheological measurements was 10wt% MG/oil. All tests were carried out using fresh samples which were pre-sheared in the rheometer with a stress of 10 Pa while kept in the isotropic phase at 70°C for 30 min.[29,30] When the MG/oil were heated to an isotropic phase, all previous orientations are removed to ensure that the samples all have the same thermal history. The temperature ramp test involved observing the rheological transformations at the boundary of phase transitions. Measurements were performed under low amplitude oscillatory shear at a low frequency of 1 rad/s with an initial applied strain amplitude 0.05%. These test conditions were well within the linear viscoelastic range as determined by stress sweeps at 70°C and 26°C. Samples were steadily cooled from 70°C to 20°C at stepped cooling rate of 1.0°C/min and the evolution of the loss and storage moduli was monitored.

3   Phase diagram

Before the discussions of completely water-free environment (the main subject of this paper), MG/oil mixtures with different concentrations of water wil be discussed here. MG/water/oil systems were produced by vigorously mixing a hot oil-MG solution with distilled water at temperature of 80°C for 20mins. For this study, the samples always contained 10% (w/w) MG in oil but different concentrations of water were applied. Calorimetric results showing the changes of the phase sequence on adding water are shown in Fig.1. Below 0.5% (w/w) of water in the MG/oil system there were no significant changes in the DSC cooling scans. Above the water concentration of 0.5% (w/w) the low-temperature transition shifted from 36°C to 18°C and the shape of the peaks became increasingly sharp. In order to understand this data, the mol concentrations were calculated in the following: 10wt% MG corresponded to 0.2793M (mol/L) and 0.5wt% water corresponds to 0.277M (mol/L). Therefore, the change in phase behavior occurs when there is, crudely, one water molecule for each molecule of MG. When the ratio of water/MG molecular numbers was above one, the changes of phase behaviors was significant.
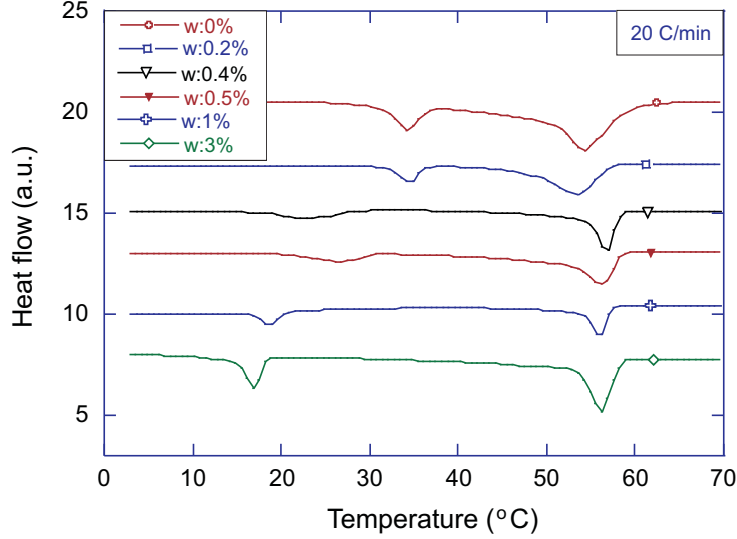
Figure 1: Above a critical concentration of water, 0.5% (w/w) the crystallization temperature T_K decreases from 36°C to 18°C. The molar estimate shows that this is the concentration at which there is one water molecule for each molecule of MG.

Water has a strong affinity to the glycerol groups and, therefore, would forming a thin layer inside the inverse lamellar ordering. We suggest that at sufficient amount of this "internal hydration", the packing of glycerol heads is disturbed, which causes the Krafft temperature shift to a lower value. However, further detailed studies of MG/oil/water systems are needed to confirm this hypothesis. Here the result simply highlights the fact that even small amounts of water in MG/oil would dominate the phase behavior of mixtures. At the same time, Fig.1 shows that if we keep the water content below 0.2% (w/w), the system may be considered water-free for all practical purposes. This is the system we discuss from now on.

DSC experiments were performed on samples of different concentrations of MG/oil, with the typical results collated in Fig.2. Based on this result, a phase diagram could be sketched, Fig.3. Similar to the results found in the MG/water solutions, two transition peaks are observed between 0°C and 100°C. In our case, the high-temperature transition corresponds to the gelation temperature of the inverse lamellar phase, and the second (low-temperature) transition represented the Krafft temperature T_K at which the aliphatic chains crystallize in the lamellae. Therefore if a sample of 10% w/w MG in oil was cooled from 100°C, the isotropic fluid phase remained until the temperature reached 60°C, when an inverse lamellar ordering was formed. In this phase, due to the effective pressure from the bilayer, the glycerol head-groups arrange with each other in a closely-packed manner and force the alkyl chains packing to the dense-brush configuration on the outside of the bilayer. The dense polymer blush arrangement is important feature in this discussion: it causes the physical properties of inverse lamellar phase to be very different with the usual lamellar ordering. In our case the grafting (head-group) plane of this brush has crystallographic order and thus does not fluctuate as the fully hydrated monolayer of glycerol head on the outside of the ordinary bilayer lamella. This, combined with the aliphatic
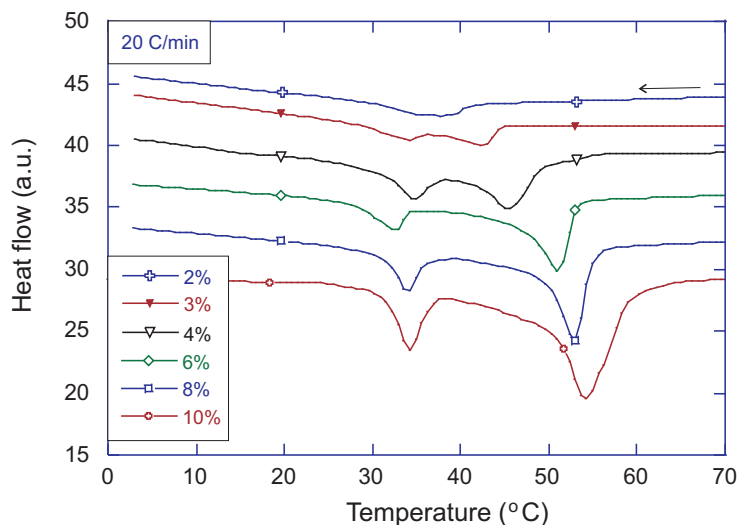
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Figure 2: Collated DSC scans of MG C18 in hazelnut oil, at varying concentrations labeled on the plot, at the cooling rate 20°C/min. There are two sequential phase changes, the first – of the inverse-lamellar transition and the second – the crystallization transition. Below 2wt%, the window of the molten lamellar phase disappears as its transition dropped below the Krafft temperature.

tails being fully extended, results in the gel-like macroscopic properties of the inverse lamellar phase.

When the material is further cooled below 36°C, the temperature of demixing was reached. The alkyl chains arrange in parallel sheets to form the structure analogous to what is usually called "sub-alpha" crystalline phase in MG/water solutions. By identifying the transition temperatures at different concentrations of MG, the phase diagram of concentration-temperature could be determined. From the phase diagram we find that increasing the MG concentration shifts the gelation (inverse-lamellar) transition upwards. However, the Krafft temperature for crystallizing alkyl chains is essentially independent of the concentration of the MG.

Thermal hysteresis is the difference between superheating and supercooling temperatures of a first-order phase transition, reflecting the metastability and the height of thermodynamic barrier between the two phases. Hysteresis of both the isotropic-lamellar and the crystallization transitions in our system is illustrated in Fig.4. The thermal hysteresis of isotropic-lamellar transition was significant and strongly depended on the heating/cooling rate, increasing when the rate of heating/cooling increased. The crystallization transition had a much weaker hysteresis, under 1°, which practically did not change at different heating or cooling rates that we could apply to this system.

It is important to verify the universality of these findings. For this purpose, a model oil, n-tetradecane, and pure surfactant MG C16 have also been considered to compare with the commercial (not perfectly pure) MG C18 and hazelnut oil. Based on many industrial applications, hazelnut oil was a natural choice for our research target. However due to a complicated
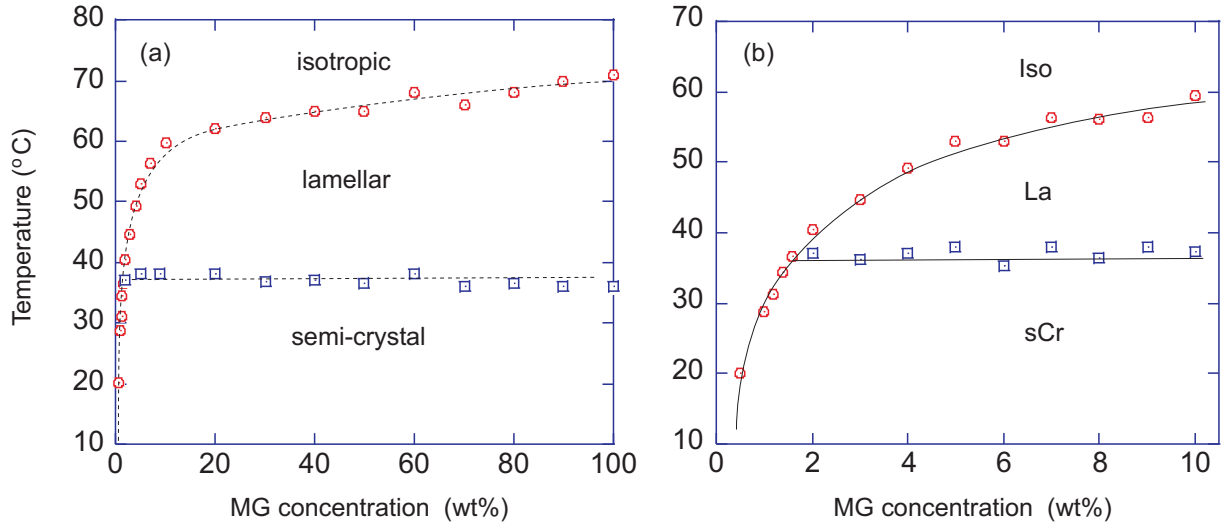
Figure 3: The phase diagram of C18 in hazelnut oil, from the DSC data. We show boundaries of the three phases across the whole range of concentrations (a). A more detailed study at low concentrations identifies the phases as: isotropic fluid, inverse lamellar and the sub-alpha crystalline phases. Below ~ 2wt% the carbon chains of MG crystallize directly.
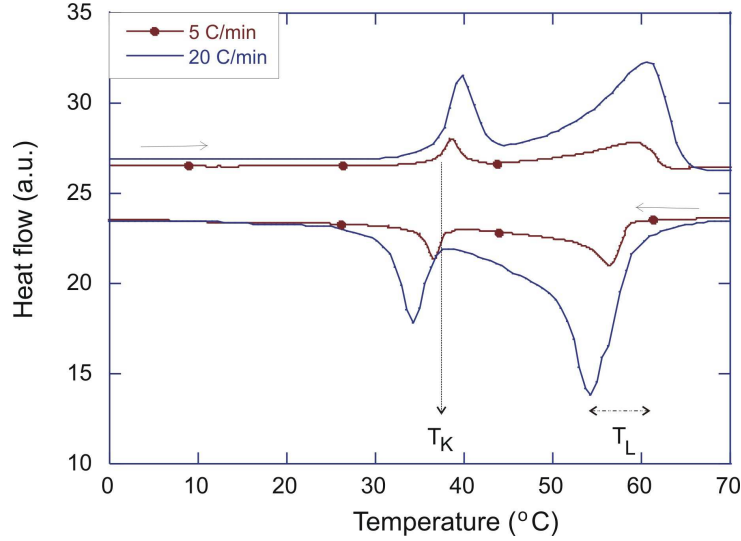

Figure 4: Heating and cooling DSC scans of 10% (w/w) MG/oil system, obtained at the rates of 20°C/min (solid line) and 5°C/min (circles). Since the transition points are defined at the onset of each calorimetric peak in the respective directions of temperature change, we conclude that hysteresis is significant during the isotropic-lamellar transition, T_{L}, but is weak during the lamellar-crystallization T_{K}.
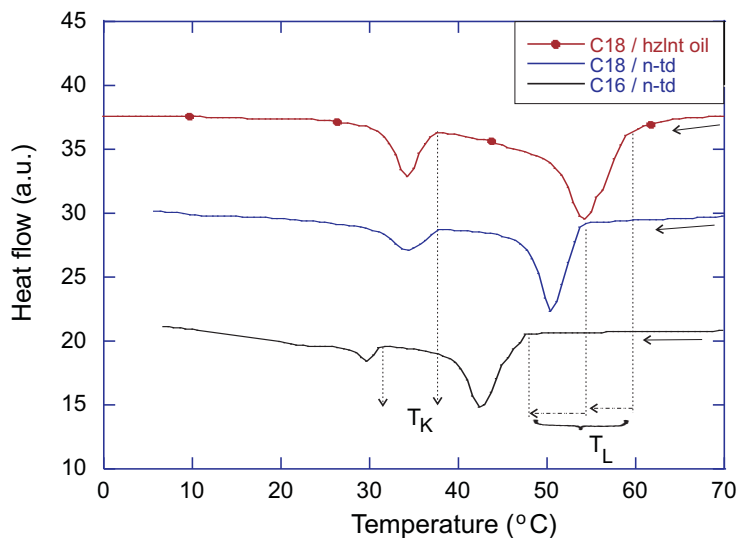
Figure 5: Illustration of the universality of the two transitions sequence at the cooling rate 20°C/min (the mixtures are labeled in the plot). Clearly the same phase sequence was observed in all systems. A small shift of the gelation temperature T_L of C18 in the hazelnut oil was observed; a shift to lower temperatures was found for both transitions for smaller molecule of C16.

mixture of different fatty acids in hazelnut oil, it is necessary to test a pure hydrophobic solvent to confirm that the results from hazelnut oil are acceptably generic for a wider range of hydrophobic solvents. The comparison of phase transformation between 10wt% MG/hazelnut oil and 10wt% MG/n-tetradecane is given in Fig.5. The transition sequence was indeed very similar. As we expected, due to the presence of small quantities of MG in the hazelnut oil itself, the gelation temperature in hazelnut oil background shifted to slightly higher values. This is consistent with the phase diagram results above, in which a higher MG concentration would shift the gelation temperature upwards. Also as expected, the Krafft crystallization temperature did not change at all This suggests that the results reported here could be used as a guide for many other oil solvents. The transitions of pure MG C16 in n-tetradecane also shown in Fig.5 to compare with the commercial MG C18. The result shows that the gelation temperature to form the inverse lamellar phase decreased, as did the Krafft temperature, by the comparable amount – which is indeed expected due to the shorter aliphatic tail in C16. This evidence suggests that MG with different length of carbon chains still retains the same features of phase transitions and confidently concluds that two crystallization peaks observed in our thermograms did not occur due to impurities or mixing of different monoglycerides.

4 Structure and rheology

The mixtures of MG in oil show diverse structuring in different phases; these could be fingerprinted in X-ray diffraction patterns in Fig.6 and Fig.7 To compare with several important

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studies in the aqueous systems we collated data from various sources to highlight the difference between oil-based and aqueous systems by indicating the X-ray peak positions in the Table1. Our X-ray scattering results show that between the gelation and the Krafft temperatures, MG molecules aggregated in the inverse lamellar bilayers surrounded by oil, with hexagonal close-packed ordering of surfactant heads in the middle plane. Below the Krafft temperature, the inverse lamellar phase was crystallized and transformed into a sub-alpha crystal form, which contained orthorhombic packing of aliphatic chains.

Table 1: Comparison of X-ray diffraction data for MG/oil and MG/water.

Systems	Long spacings (Å)	Short spacings (Å)
MG/water(Lamellar)	48.5Å	-
MG/water(alpha crystal)	54.3Å	4.18Å
MG/water(beta crystal)	48.5Å	4.60-4.38-4.31-4.04Å
MG/oil(Inverse lamellar)	52Å	4.17-4.11Å
MG/oil(sub-alpha crystal)	49Å	4.27-4.17-4.06-3.95-3.79-3.62Å

Above the gelation temperature (57°C) the sample was in an isotropic phase with no X-ray scattering features and the powder diffraction pattern similar to the one from the pure hazelnut oil. At 45°C, when the concentration of MG was above 2wt%, the inverse lamellar phase appeared. In this phase MG heads packed together with 2D hexagonal ordering and forced the carbon chains to extend due to dense lateral confinement, to from several lamellar plates. Above a 4wt% concentration of MG, these plates formed a firm but brittle gel network capable to hold the oil inside. Although Fig.6 reports only on the 10wt% sample, we have seen the same lamellar spacing scattering in all such systems, but with much lower intensity making it more difficult to present. A series of concentric rings in small-angle region was sufficient to determine the lamellar ordering. After subtracting the oil background, Fig.7 we find a series of diffraction peaks with the ratio of spacing following the sequence of 1, 1/2, 1/3, 1/4. These peaks represented successively higher-order reflections from the periodic lamellar structure and were in good agreement with the expected thickness of the lamellar bilayer, determined as around 53Å.

At 40°C, in the inverse lamellar phase, twin X-ray diffraction peaks were observed in the wide-angle scattering region. This is an important feature. The two rings indicate the regular spacings at 4.17Åand 4.11Å, which in fact characterize the spacings between neighboring glycerol heads. Within the same layer, the 4.17Åspacing is characteristic of a 2D (dense-packing) hexagonal order, while the 4.11Åline corresponds to the distance between glycerol heads in the two neighboring planes inside the bilayer.

Below the Krafft temperature, at 35°C, the sub-alpha crystalline phase appears. In this case the structure were still characterized by inverted lamellar bilayers, but with the thickness of each bilayer slightly reduced to 50Åas indicated by small-angle scattering lines. The orthorhombic crystallized chain packing pronounced sequence of wide angle diffraction peaks, with a strong line at 4.17Åand several weaker peaks between 4.27 and 3.62Å. Obviously this phase, which we continue to call "sub-alpha crystal" is not the well known anhydrous crystalline form, beta-crystal (with an orthogonal subcell), which would be characterized by the sharp wide angle
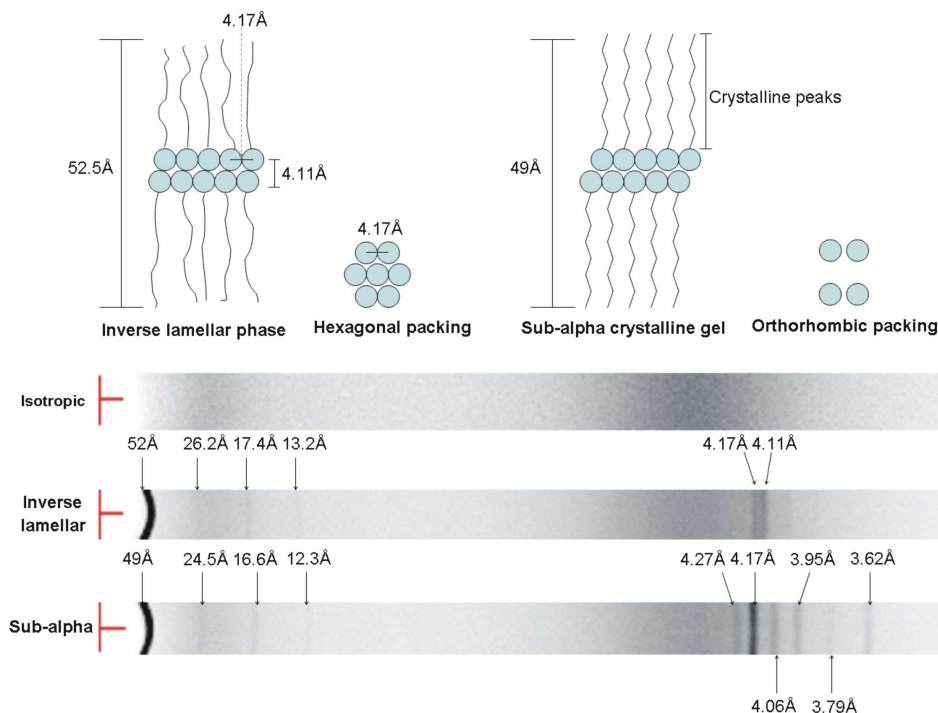
Figure 6: In the isotropic phase, there are no significant ordering and the scattering pattern represents just the oil background. In the inverse lamellar phase and sub-alpha crystalline phases, the concentric rings in the small-angle region confidently describe the lamellar ordering. In the inverse lamellar phase the twin wide-angle peaks could be observed at 4.17Åand 4.11Å. They corresponded to the hexagonal packing of in-plane glycerol heads and the ordering of plane-plane glycerol heads, which cannot be found in the ordinary hydrated lamellar phase. The sub-alpha crystalline phase was in orthorhombic chain packing which could be characterized by a series of peaks between 4.27 and 3.62Å, with a strong wide-angle peak at 4.17Åstill reflecting the 2D dense packing of head-groups.

peaks at 4.60-4.38-4.31-4.04Å.[15]

Rheological experiments were carried out in the linear viscoelastic region. The measurement involved observing the low frequency shear modulus changes as a function of temperature, as the system evolved from isotropic to the inverse lamellar, and further to the sub alpha crystalline phases. This provided information about macroscopic mechanical rigidity of the systems. The materials were heated to 80°C, well into the isotropic phase, and pre-sheared at 10Pa for 30min to erase the thermal and mechanical history. The samples were then cooled through two phase transition zones at a chosen well controlled rate (1°C/min). A typical result is illustrated in Fig.8. The plot clearly shows three phases. In the high-temperature region, the sample was in isotropic fluid phase; the loss modulus G'' is higher than the storage modulus G' and both are in the range of 10^{-2} Pa (we have not specifically focused on that liquid region and the data is noisy due to the low modulus values). When the temperature drops down below
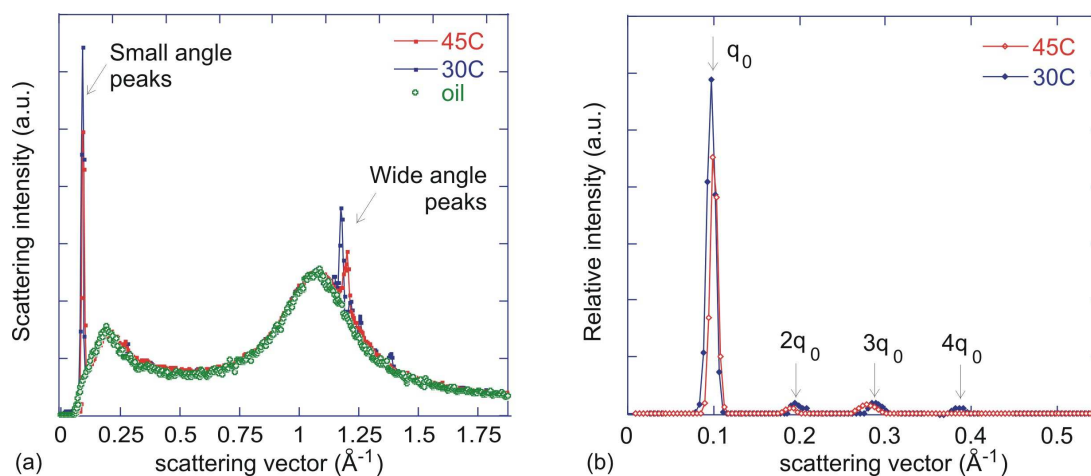
Figure 7: The cross section intensity scans of X-ray diffraction patterns. (a) The original X-ray diffraction includes the broad band background of the hazelnut oil solvent. (b) By subtracting the oil background, the resulting small-angle peaks are clear and show the high-order reflections at correct positions reflecting the lamellar bilayer structure. The similarity of the layered structures in the inverse lamellar phase and the sub alpha crystal is also evident.
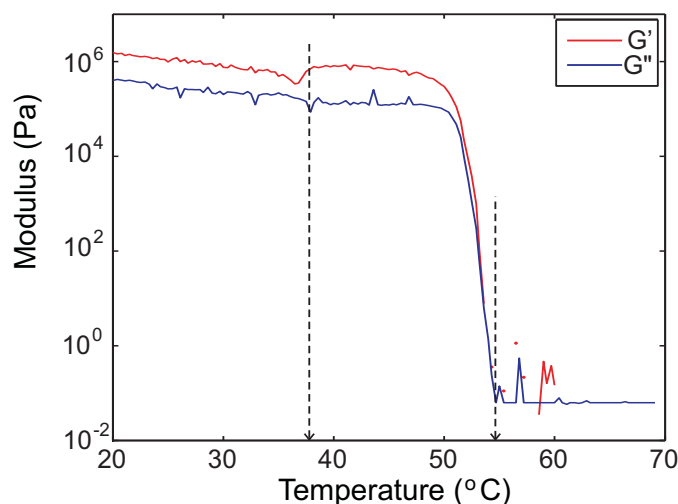

Figure 8: There are three regions in a low-frequency rheological behavior of 10wt% MG/oil which correspond to the three phases. Above 57°C the sample is in the isotropic fluid phase. From 57°C to 38°C, the modulus increased to 10^3Pa while the inverse lamellar ordering consolidated. After the crystallization temperature of 38°C was reached, a small drop of the moduli at the phase transition is registered; apart from that, the rheological response of an elastic gel was similar to the lamellar phase.

the isotropic-lamellar transition point T_L, the rapid increase in mechanical rigidity is immediately expressed by the storage modulus. The system acquires mechanical characteristics of a gel. On continuously cooling down towards the crystallization temperature T_c, a region of pre-transitional effect was observed, as is common during nucleation at first-order transitions and reflecting the increase in dissipation in a fluctuating system. Below the crystallization transition the sample is in sub-alpha crystalline phase. Because the percolating network structure has kept its shape to encapsulate the liquid oil inside the lamellar scaffold, the mechanical response in this phase did not show much difference with the inverse lamellar phase.

5 Conclusion

To the best of our knowledge, this is the first systematical study of the phase behavior of MG/oil systems. Calorimetric, X-ray, and rheometric data gathered in this work yield a comprehensive set of macroscopic and microstructural characteristics. First of all, we identified the significant change in properties by adding water to the MG/oil system and outlined the boundary (of water content) below which the system can be considered non-aqueous. Therefore MG/oil solution should be distinguished from any water-containing MG system. The phase diagram in concentration-temperature variables, determined by DSC, showed the three essential MG/oil phases in the whole region of the surfactant concentrations. These phases were separated by two lines of first order phase transitions; the lower transition line being the Krafft temperature, independent of surfactant concentration. The thermal hysteresis of the two transitions was tested at different heating/cooling rates. We found that lamellar transition had a significant thermal hysteresis, but the Krafft temperature did not. These conclusions were quite general, since the study of MG in two very different oil presented very similar phase behavior. The measurements of phase transitions of MG C16/n-tetradecane generalized the result to different carbon-chain length of MG and clearly suggest that the sequence of the two transitions was not arising from the impurities of MG but based by the generic phase ordering of MG in hydrophobic fluid matrix.

X-ray diffraction proved the existence of two phases, both characterized by the rigid lamellar network spanning the whole volume: the inverse lamellar, and the sub-alpha crystalline phase. The most important finding is that in the inverse lamellar phase the glyceride groups are densely packed in the hexagonal manner in the planes in the middle of bilayers. This explains the two "twin" wide-angle reflections corresponding to spacings of 4.17Åand 4.11Å, which characterize the regular 2-dimensional packing of in-plane and plane-plane surfactant heads. Due to the orderly hexagonal packing of glycerol heads, the carbon chains are forced to form the dense brush ordering. Due to this added rigidity of lamellar bilayers, the rheological behavior of the inverse lamellar phase was similar with the gel-like materials known at lower temperatures in the aqueous systems. Below the crystallization point, the lamellar phase transforms into the sub-alpha crystalline phase, which has orthorhombic packing of aliphatic chains. There is no significant change in the rheological response of a gel in this phase.

Apart from the detailed results and characterization of phases, the important message of this work is that although the phase sequence of MG ordering in oil is superficially similar to that

in water, there are important differences in the phase structure, both in the lamellar and in the low-temperature crystalline phases. The origin of these differences lies in the inverted lamellar nature, which (in contrast with the hydrated lamellae) does not allow sufficient fluctuations of glycerol heads and results in much higher ordering even in the lamellar phase. It also leads to the reversal of order between crystallization of heads and aliphatic tails, so the first crystalline phase that appears below the lamellar is the "sub-alpha" phase (which exists much lower down the phase sequence in aqueous systems). In this work we only studied the phases that establish immediately, on in a short time after cooling through phase transitions. A subsequent paper will focus on the effects of long-time aging in both phases, and the nature of the global equilibrium order of MG in hydrophobic environment, which is controlled by intra- and inter-glycerol hydrogen bonding of monoglyceride.

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